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Assistant Commissioner of Patents
Washington, D.C. 20231

TRANSMITTAL OF TRANSLATION OF PRIORITY DOCUMENT

Transmitted herewith for filing in the above-referenced patent application are the following documents:

1. Verification of Translation of Priority Document of Japanese Application No. 10 (1998)-366719, filed December 24, 1998 together with a copy of the Specification, Sequence Listings, Figures, Claims and Abstract;
2. This Transmittal Letter; and
3. Return Postcard.

Applicant(s) believe that no fees are required. However, if for any reason a fee is required, the Commissioner is hereby authorized to charge any fees to Deposit Account No. 04-1105.

If the enclosed papers are considered incomplete, the mailroom and/or the Application Branch is respectfully requested to contact the undersigned at (617) 439-4444, Boston, Massachusetts.

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on February 28, 2002.

By: _____
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Date: February 28, 2002

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Respectfully submitted,

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VERIFICATION OF TRANSLATION

I, the undersigned Masaaki Iwabuchi, translator, having an office at Abe, Ikubo & Katayama, 2-8-7, Yaesu, Chuo-ku, Tokyo, Japan, declare that I am well acquainted with the Japanese and English languages, and that the attached English text is, to the best of my knowledge, a complete and accurate translation from the Japanese text of the priority document, Japanese Patent Application No. 10 (1998)-366719 (Ref. No. A98234), dated December 24, 1998.

The undersigned further declares that all statements made herein of his/her own knowledge are true, and all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like, so made, are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Date: February 13, 2002

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Signature of Translator

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[Document] SPECIFICATION

[Title of the invention] UCP-2 Promoter and Its Use

[Claims]

[Claim 1] A DNA containing uncoupling protein-2 (UCP-2) promoter region containing the regulator sequence.

[Claim 2] A DNA described in claim 1 wherein the regulator sequence is a sequence containing peroxisome proliferator response element (PPRE).

[Claim 3] A DNA described in claim 1 wherein the regulator sequence is a sequence containing CCAAT/enhancer binding protein (C/EBP) binding sequence.

[Claim 4] A DNA described in claim 1 wherein the promoter region is a base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a base sequence containing a part of the said base sequence.

[Claim 5] A DNA described in claim 1 wherein the DNA is a recombinant vector containing a DNA containing a structural gene under control of UCP-2 promoter region containing a regulator sequence.

[Claim 6] A transformant transformed by a DNA described in claim 1.

[Claim 7] A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in claim 6.

[Claim 8] A method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of a transformant described in claim 6.

[Claim 9] A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in claim 6.

[Claim 10] A pharmaceutical composition comprising a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using a screening method described in claim 7 or a screening kit described in claim 9.

[Detailed description of the invention]

[0001]

[Technical field to which the invention pertains]

This invention relates to a novel promoter for gene expression and its use. Specifically, this invention relates to a DNA containing the promoter region of human uncoupling protein-2 (UCP-2) gene, a transformant transformed with the said DNA, and a method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity.

[0002]

[Prior art]

Uncoupling protein (UCP) is a proton transporter present in the mitochondrial inner membrane. Since UCP changes intracellular energy stored as fat to heat without using other energy consuming processes, UCP is considered to play an important role in maintenance of body temperature in homeothermal animals. Because of this function, UCP is considered to be an important factor that determines the efficiency of energy metabolism in homeothermal animals.

Three molecular species of uncoupling protein have been identified to date, and are called uncoupling proteins-1 (UCP-1), -2 (UCP-2 or UCPH), and -3 (UCP-3).

UCP-1, the first isolated among the uncoupling protein family, is specifically expressed in brown fat cells (Line, C.S. and Klingerberg, M. (1980), FEBS Lett., 113, 299-303; Jacobsson, A. et al. (1985), J. Biol. Chem., 260, 16250-16254; Bouillaud, F. et al. (1986), J. Biol. Chem., 261, 1487-1490). UCP-2 was isolated as a homologue of UCP-1, and confirmed to be widely expressed in various organs (Gimeno, R.E. et al. (1997), Diabetes, Vol. 46, 900-906; Fleury, C., et al. (1997), Nature Genet., Vol. 15, 269-272). UPC-3 was isolated as a UCP specifically expressed in muscle (Vidal-Puig, A. et al. (1997), Biochem. Biophys. Res.

Commun., Vol. 235, No. 1, 79-82; Boss, O. et al. (1997), FEBS Lett., 408, 33-38).

Generally, UCP-1 is considered to play an important role in maintenance of body temperature in rodents and hibernants. As for the basis, the number of brown fat cells that mainly express UCP-1 is lower in large sized animals and animal species inhabiting in relatively warm weather (Rothwell, N.J. and Stock, M.J. (1979), Nature, Vol. 281, 31-35). Thus, in these animals including human, UCP-2 or UCP-3, not UCP-1, may mainly be responsible for the control of the normal body temperature maintenance system and energy consuming process (Hosoda, K. et al. (1998), Obesity Research (Himan Kenkyu), Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), Nature, Vol. 387, 90-93).

Therefore, it may be possible to adjust the energy consumption/accumulation balance by controlling the gene expression or activity of UCP-2 or UCP-3 in these animals including human (Hosoda, K. et al. (1998), Obesity Research (Himan Kenkyu), Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), Nature, Vol. 387, 90-93). In human, enhancement of energy consumption is considered to promote consumption of not only dietary energy but also energy accumulated as fat. Accordingly, a decrease of body fat in human may lead to improvement of obesity, the major cause of lifestyle diseases which become a problem in developed countries in recent years (Fleury, C. et al. (1997), Nature Genetics, Vol. 15, 269-272).

UCP-2 is also considered to be the major cause of fever observed in immunological inflammation such as infection, and inhibition of UCP-2 gene activity may reduce fever in immunological inflammation (Shigenaga, F.R. et al. (1998), Biochiem. Biophys. Res. Commun., Vol. 244, No. 1, 75-78).

In animals, especially in higher animals, organs differentiate and mature upon biogenesis, and develop to exert each function. During this process, various organ-

specific proteins are transiently or constantly expressed and provide the organ-specificity.

The general gene expression control system in animals includes the transcription induction system (promoter, enhancer). Promoter regions are generally located adjacent to the 5′ upstream region of base sequences on chromosomes that are normally transcribed into messenger RNAs. Transcriptional regulator protein is bound to or dissociated from base sequence generally called regulator sequence in promoter regions, by which the transcription level of genes located downstream of the 3′ region is regulated. Therefore, the transcriptional gene expression level can be estimated from the promoter activity to some extent. It is also known that the base sequences located downstream of the 3′ region of a promoter do not affect the promoter activity in most cases. Therefore, promoter activity can be readily measured by substituting the transcribed messenger RNA for a base sequence encoding a protein with detectable enzyme activity (reporter). Recent technical innovation has made measurement of promoter activity using reporters very sensitive and simple, and measurement of promoter activity is used in drug screening and analysis of biological function.

For example, transcriptional regulatory factors of fat cell differentiation include peroxisome proliferation-activated receptor γ (PPAR γ) (Tontontz, P. et al. (1995), Curr. Opin. Genet. Dev., Vol. 5, 571-576), retinoid X receptor (RXR), CCAAT/enhancer binding protein (C/EBP) (Cornelius, P., et al. (1994), Annu. Rev. Nutr. Vol. 14, 99-129), etc. The transcriptional regulation by these factors are closely involved in the gene expression related to fat cells. It has been reported that the promoter regions of fat cell-related genes including UCP-2 gene contain the binding sequences for these transcriptional regulatory factors (regulator sequences). These sequences in promoters are considered to play important roles in the

actual regulation of UCP-2 transcription in vivo.

[0003]

[Problem to be solved by the invention]

Accordingly, substances that enhance expression of UCP-2 or UCP-3 gene and protein may be used as antiobestic drugs that reduce body fat content. UCP-2 is also considered to be the major cause of fever in immunological inflammation observed in infection, and substances that inhibit UCP-2 gene activity may reduce fever in immunological inflammation.

If a cell line expressing an appropriate reporter gene connected to the promoter region described above is established, the cell line may be used for screening a drug that promotes or inhibits the UCP-2 expression. In screening substances that may be used as antiobestic drugs, responses more similar to those in vivo can be obtained by including these regulator sequences in the promoter-reporter system, which is very advantageous in screening human antiobestic drugs.

However, human UCP-2 promoter containing the regulator sequence has not yet been identified, and no simple screening method using the promoter described above has been available for substances that affect the human UCP-2 gene expression.

[0004]

[Means for solving problem]

The inventors performed extensive studies, and successfully obtained the human genomic UCP-2 gene using human UCP-2 cDNA fragments as probes in attempt to establish a screening method for searching substances that affect the human UCP-2 gene expression. The gene was digested with restriction enzymes, and 6.5 kb DNA of the upstream region containing a part of the structural gene encoding UCP-2 was obtained. From the DNA obtained, 3.5 kb

DNA containing the base sequence deduced to be the 1st and 2nd exons (2.5 kb DNA as the 5' upstream region) were re-cloned in plasmid DNA.

A plasmid DNA was constructed by connecting luciferase gene as a reporter gene to downstream of the 3.5 kb DNA. Measuring the luciferase activity in transformants of HepG2 cells, UCP-2 promoter was found in the 3.3 kb DNA of the upstream region of the UCP-2 structural gene. As a result of detailed analysis, the regulator sequence that may control the expression of UCP-2 was found.

The inventors proceeded the study based on these findings, and completed the present invention.

[0005]

The present invention relates to the followings:

(1) A DNA containing uncoupling protein-2 (UCP-2)promoter region containing the regulator sequence;

(2) A DNA described in (1) wherein the regulator sequence is a sequence containing peroxisome proliferator response element (PPRE);

(3) A DNA described in (1) wherein the regulator sequence is a sequence containing CCAAT/enhancer binding protein (C/EBP) binding sequence;

(4) A DNA described in (1) wherein the promoter region is a base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a base sequence containing a part of the said base sequence;

(5) A DNA described in (1) wherein the DNA is a recombinant vector containing DNA having a structural gene under control of UCP-2 promoter region containing a regulator sequence;

(6) A transformant transformed by a DNA described in (1);

(7) A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in (6);

(8) A method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of a transformant described in (6);

(9) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in (6); and

(10) A pharmaceutical composition cntaining a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using a screening method described in (7) or a screening kit described in (9).

[0006]

[MODE OF EMBODIMENT OF THE INVENTION]

A DNA containing the UCP-2 promoter region containing the regulator sequence of this invention may be any DNA containing the regulator sequence described below with UCP-2 promoter activity.

Specifically, a DNA of this invention may be any DNA containing the base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a part of said sequence.

A DNA of this invention may be (i) genomic DNA, (ii) cDNA, and synthetic DNA derived from human cells (e.g. hepatocytes, splenocytes, neurocytes, glial cells, pancreatic β cells, bone marrow cells, mesangium cells, Langerhans' cells, epidermal cells, epithelial cells, endothelial cells, fibroblasts, fibre cells, muscle cells, fat cells, immune cells (e.g. macrophages, T cells, B cells, natural killer cells, mast cells, neutrophils, basophils, eosinophils, monocytes), megakaryocytes, synovial cells, chondrocytes, osteocytes, osteoblasts, osteoclasts, mammary cells, and interstitial cells, or precursor cells, stem cells, or cancer cells of said cells, and any tissue in which said cells are present, for example, the brain, each region of the brain (e.g. olfactory bulbs, amygdalloidal nucleus, basal ganglia, hippocampus, thalamus, hypothalamus,

cerebral cortex, medulla oblongata, cerebellum), spinal cord, pituitary gland, stomach, pancreas, kidneys, liver, gonads, thyroid gland, gallbladder, bone marrow, adrenal glands, skin, muscle, lung, digestive tract (e.g. large intestine, small intestine), blood vessels, heart, thymus, spleen, salivary glands, peripheral blood, prostate, testes, ovaries, placenta, uterus, bones, cartilages, joints, and skeletal muscles.

Specifically, a recombinant DNA containing the human UCP-2 promoter region of this invention can be obtained as follows.

Using the base sequence corresponding to the previously reported amino acid sequences of human UCP cDNA (Fleury, C. et al. (1997), Nature Genet. Vol. 15, 269-272) as the probes, for example, human genomic library inserted in EMBL3 vector is screened by publicly known method, and λ phage clones that react with the probes are obtained. A DNA is extracted from these phage clones, and the restriction enzyme map of the human gene inserted in the clones is prepared. DNA fragments are prepared by digestion with restriction enzymes, and the fragments react with the probes for the most upstream region of the cDNA are re-cloned in vectors for animal cells such as pCD vector, cDM8 vector (Aruffo, A. and Seed, B. (1987), Proc. Natl. Acad. Sci. USA, 84, 8573-8577), and retrovirus vector (Cone, R.D. and Mulligan, R.C. (1984), Proc. Natl. Acd. Sci. USA, 81, 6349-6353), and *Escherichia coli* plasmids such as pUC vector (Vieira, J. and Messing, J. (1987), Methods in Enzymology, 153, 3-11), and pCR-blunt vector (Ausubel, F.M. et al. (1994), Current Protocols in Molecular Biology), but not limited to these vectors. The base sequences of the cloned DNA are determined, and the position of the translation initiation codon on the gene can be determined by, for example, comparing the base sequence with the cDNA sequence. The position of the transcriptional initiation site on the gene can also be determined by comparing the

base sequence with the 5' end of known cDNA. By investigating motifs in the entire sequence, the binding site of known transcriptional regulatory factors can be determined.

The obtained DNA can be used without modification or if necessary, after digestion with restriction enzymes or being bound by linkers.

To measure the promoter activity, a detectable structural gene may be connected in downstream of the promoter region. For the structural gene connected in downstream of the promoter region, various reporter genes are used. For the reporter gene, luciferase, chloramphenicol acetyltransferase (CAT) gene, alkaline phosphatase gene, and β-galctosidase gene are commonly used, but any other structural genes for which a method of detecting the gene product is available may be used. To insert the above structural gene into the vector, the structural gene is ligated to an appropriate restriction enzyme site located downstream of the promoter region in the correct transcriptional orientation.

[0007]

For the host transformed by the recombinant vector described above, for example, *Escherichia genus*, *Bacillus genus*, Yeast, insect cells, insects, and animal cells are used.

Specific examples of the host *Escherichia genus* are *Escherichia coli* K12·DH1 [Proceedings of the National Academy of Sciences of the USA (Proc. Natl. Acad. Sci. USA), Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JM109, JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)], and C600 [Genetics, Vol. 39, 440 (1954)].

For the host *Bacillus genus*, for example, *Bacillus subtilis* MI114 [Gene, Vol. 24, 255 (1983)] and 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)] are used.

For the host yeast, for example, *Saccharomyces cerevisiae* AH22, AH22R$^-$, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, and *Pichia pastoris* are used.

For the host insect cells, for example, when the virus is AcNPV, Spodoptera frugiperda cells (Sf cells), MG1 cells derived from the middle gut of Trichoplusia ni, High Five™ cells derived from Trichoplusia ni eggs, Mamestra brassicae-derived cells, and Estigmena acrea-derived cells are used. When the virus is BmNPV, silkworm-derived cell line Bombyx mori N (BmN cells) are used. For said Sf cells, for example, Sf9 cells (ATCC CRL1711), Sf21 cells (Vaughn, J.L. et al., In Vivo, 13, 213-217 (1977)) are used.

For the host insect, for example, silkworm larvae are used [Maeda et al., Nature, Vol. 315, 592 (1985)].

For the host animal cells, for example, monkey COS-7 cells, Vero, Chinese hamster CHO cells (CHO), dhfr gene-deficient Chinese hamster cells CHO (CHO (dlfr$^-$) cells), mouse L cells, mouse AtT-20, mouse myeloma cells, rat GH3, human FL cells, white fat cells, egg cells, ES cells (Evans, M.J. and Kaufman, K.H. (1981), Nature, 292, 154) are used.

Animal cells, especially white fat cells, may be used. As a process of DNA transfer to individual animals, egg cells and ES cells (Evans, M.J. and Kaufman, K.H. (1981), Nature, 292, 154) are used.

For the method of transforming these cells, the calcium phosphate method (Graham et al. (1973), Virology, 52, 456), electroporation (Ishizaki et al. (1986), Cell Engineering (Saibo Kogaku), 5, 577), and microinjection are used.

More specifically, for transformation of bacteria of *Escherichia genus*, for example, the methods published in Proc. Natl. Acad. USA, Vol. 69, 2110 (1972) and Gene, Vol. 17, 107 (1982) are used.

Bacteria of *Bacillus genus* can be transformed following, for example, the method published in Molecular &

General Genetics, Vol. 168, 111 (1979).

Yeast can be transformed following, for example, the methods published in Methods in Enzymology, Vol. 194, 182-187 (1991) and Proc. Natl. Acad. USA, Vol. 75, 1929 (1978).

Insect cells and insects can be transformed following, for example, the method published in Bio/Technology, 6, 47-55 (1988).

Animal cells can be transformed by, for example, the methods described in Cell Engineering (Saibo Kogaku), Separate Vol. 8, New Cell Engineering Experimental Protocol, 263-267 (1995) (Shujun-sha) and Virology, Vol. 52, 456 (1973).

The transformant described above is cultured in the presence of the specified compound, and by measuring and comparing the gene product in the cultured material, the ability of controlling the promoter activity of the compound can be examined.

[0008]

The transformant is cultured by publicly known methods. For the medium for culturing the transformant using *Escherichia* and *Bacillus* hosts, liquid medium is appropriate, which contains carbon source, nitrogen source, inorganic compounds, and other substances necessary for the growth of the transformants. The carbon source includes, for example, glucose, dextrin, soluble starch, and sucrose, etc. The nitrogen source includes, for example, inorganic and organic compounds such as ammonium salts, nitrates, cornsteep liquor, peptone, casein, meat extract, soybean cake, and potato extract, etc. The inorganic compounds include, for example, calclium chloride, sodium dihydrogen phosphate, and magnesium chloride, etc. Yeast extract, vitamins, and growth-stimulating factors may be added. The pH about 5 - 8 is desirable for the culture medium.

For the culture medium for bacteria of *Escherichia genus*, for example, M9 medium containing glucose and casamino acid (Miller, Journal of Experiments in Molecular

Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972) is preferred. When a higher efficiency of the promoter is required, reagent such as 3-β-indolylacrylic acid may be added. When the host is bacteria of *Escherichia genus*, the bacteria are generally cultured at about 15 - 43°C for about 3 - 24 hours, and aeration or stirring may be added to the culture if necessary.

When the host is bacteria of *Bacillus genus*, the bacteria are generally cultured at about 30 - 40°C for about 6 - 24 hours, and aeration or stirring may be added to the culture if necessary.

For the medium for culturing the transformant in yeast host, for example, Burkholder minimum medium [Bostian, K.L. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)] and SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proc. Natl. Acd. Sci. USA, Vol. 81, 5330 (1984)] are used. The pH of the medium is preferably adjusted to about 5 - 8. The culture conditions are generally about 20 - 35°C for about 24 - 72 hours, and aeration or stirring may be added to the culture if necessary.

For the medium for culture of the transformants in insect cells and insect hosts, Grace's insect medium [Grace, T.C.C., Nature, 195, 788 (1962)] containing appropriate supplements such as inactivated 10% bovine serum is used. The pH of the medium is preferably adjusted to about 6.2 - 6.4. Usually, the culture conditions are at about 27°C for about 3 - 5 days, and aeration or stirring may be added to the culture if necessary.

For the culture medium of the transformants in animal cell hosts, for example, MEM containing about 5 - 20% fetal calf serum [Science, Vol. 122, 501 (1952)], DMEM [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, Vol. 199, 519 (1967)], and 199 medium [Proceeding of the Society for the Biological Medicine, Vol. 73, 1 (1950)] are used. The pH is preferably adjusted to about 6 - 8. Usually, the culture

conditions are about 30 - 40°C for about 15 - 60 hours, and aeration or stirring may be added to the culture if necessary.

[0009]

Specifically, the regulator sequence may be any sequence of the base sequence presented by position from 1 to 2270 of SEQ ID NO: 1 to which the UCP-2 transcriptional regulatory factor can bind, such as sequences containing peroxisome proliferator response element (PPRE) presented by position 284 to 296 of SEQ ID NO: 1, sequences containing CCAAT/enhancer binding protein (C/EBP) binding sequence presented by position 1316 to 1320, 1364 to 1368, or 1698 to 1692 of SEQ ID NO: 1, sequences containing glucocorticoid response element (GRE) presented by position 753 to 758, 1023 to 1030, or 1450 to 1455 of SEQ ID NO: 1, and sequences containing MyoD presented by position 1428 to 1437 of SEQ ID NO: 1.

Therefore, a DNA of this invention contains the promoter region containing the said regulator sequence, and a DNA of this invention may contain a multiple number of the said regulator sequences.

For the base sequences containing a part of the base sequence presented by position 1 to 2270 of SEQ ID NO: 1, any base sequences containing the regulator sequence described above may be used. Specifically, the base sequence presented by position 255 to 430 of SEQ ID NO: 1, the base sequence presented by position 255 to 717 of SEQ ID NO: 1, the base sequence presented by position 717 to 1133 of SEQ ID NO: 1, the base sequence presented by position 1133 to 1389 of SEQ ID NO: 1, and the base sequence presented by position 255 to 1857 of SEQ ID NO: 1 are used.

[0010]

Since a DNA of this invention contains the UCP-2 promoter region containing the regulator sequence, using the transformant described above, a compound or its salt

that promotes or inhibits UCP-2 promoter activity can be screened. The said screening method, screening kit, and the said compound that promotes or inhibits UCP-2 promoter activity obtained using the said screening method and screening kit are specifically explained below.

(1) A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity

A transformant transformed by the DNA of this invention described above is useful for searching and determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention.

A method for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention is characterized by measuring and comparing polypeptide expression between a transformant of this invention contacted to test compound and the transformant lacking the UCP-2 promoter region of this invention contacted to the test compound.

The said test compound includes peptides, proteins, non-peptide compounds, synthetic compounds, and fermentation products, etc., and these test compounds may be novel compounds or known compounds.

For the polypeptide to be expressed, polypeptides encoded by the structural genes described above (preferably reporter genes) are used.

For the measurement method of polypeptide expression, for example, measurement of luciferase activity according to the method described by Brasier, A.R. et al. (1989) in Biotechniques Vol. 7, 1116-1122, is used.

[0011]

(2) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity

A kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production) is characterized by use of the transformant described above.

Examples of the kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invenstion are as follows.

① Screening reagents

1. Cell culture medium

Dulbbecco's modified Eagle's medium (Gibco Co.) supplemented with 10% fetal calf serum (Gibco Co.)

2. Cell differentiation medium

Dulbbecco's modified Eagle's medium (Gibco Co.) supplemented with 5% rabbit serum (Gibco Co.)

3. Plasmid for measurement of UCP-2 promoter activity

pGL3-basic (Promega Co.) plasmid DNA carrying UCP-2 promoter sequence of this invention and a structural gene (e.g. luciferase gene) inserted downstream of the UCP-2 promoter

4. Host cell line

MG-63 cells (osteosarcoma cell line, obtained from ATCC)

5. Test compounds

Aqueous solutions are stored at 4°C or -20°C, and diluted to 1 μM with cell differentiation medium at use. Test compounds that are slight soluble in water are dissolved in dimethylforamide, DMSO, and methanol.

② Screening method

Host cells are seeded in 96-well microplates at a density of 1×10^5 cells/well, and cultured in an incubator at 37°C in 5% CO_2 overnight.

The cells are transfected with 1 μg/well of plasmid for UCP-2 promoter activity measurement.

One hour after transfection, 0.1 ml of test compound is added to each well, and the cells are cultured in an incubator at 37°C in 5% CO_2 for 48 hours.

After culture, 0.1 ml of PicaGene LT (Toyo Ink Co.) is added to each well, stirred for five minutes, and then the luminescence is measured using a 96-plate measurement system (Amersham-Pharmacia Co.).

(3) A compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using the screening method described in (1) and the screening kit described in (2)

If a compound that promotes or inhibits UCP-2 promoter activity is found using the screening method described in (1) or the screening kit described in (2), the compound may be used as a prophylactic or therapeutic drug for obesity syndrome, and thus, the compound may be used as a radical therapeutic drug for lifestyle diseases (diabetes, hypertension, hyperlipidemia). Therefore, the compound may be used as an antiobestic drug, an antidiabetic drug, a depressor, and an antihyperlipemic drug.

When the compound reduces or inhibits the promoter activity, the compound may be used as an antipyretic drug because the compound decreases or inhibits heat production.

[0012]

When the said compound is used as prophylactic and/or therapeutic drugs for the diseases described above, the preparation can be obtained by the conventional methods.

For example, the said compound can be orally administered as sugar coated tablet, capsule, elixir, and microcapsule, etc., or non-orally as injection such as aseptic solution in water or other pharmaceutically acceptable liquid and suspension. Preparations can be manufactured by, for example, mixing with physiologically acceptable known carrier, flavor, filler, vehicle, antiseptic, stabilizer, and binder in a unit-dosage form required for generally approved drug preparation. The amount of the active ingredient is set to prepare an appropriate dosage within the specified range.

For the additive miscible with tablets and capsules, for example, binders such as gelatin, cornstarch, tragacanth and acacia, fillers such as crystalline cellulose, swellings such as cornstarch, gelatin, and alginic acid, lubricants such as magnesium stearate, sweeteners such as sucrose, lactose and saccharin, and

flavors such as peppermint, akamono oil and cherry are used. When the unit-dosage form is capsule, liquid carrier such as fat and oil may be contained. Aseptic compositions for injection can be formulated following the usual preparation procedure such as dissolving or suspending the active substance in vehicle, e.g. water for injection, and natural plant oils e.g. sesame oil and coconut oil. For the aqueous solution for injection, for example, physiological saline and isotonic solutions (e.g. D-sorbitol, D-mannitol, sodium hydrochloride) containing glucose and other adjuvant is used. Appropriate dissolution-assisting agents, for example, alcohol (e.g. ethanol), polyalcohol (e.g. propylene glycol, polyethylene glycol), and nonionic surfactant (e.g. polysorbate 80(TM), HCO-50) may be combined. For the oily solution, for example, sesame oil and soybean oil are used, and dissolution-assisting agents such as benzyl benzoate and benzyl alcohol may be combined.

[0013]

The prophylactic/therapeutic drugs described above may be combined with, for example, buffers (e.g. phosphate buffer, sodium acetate buffer), soothing agents (e.g. benzalkonium chloride, procaine hydrochloride), stabilizers (e.g. human serum albumin, polyethylene glycol), preservatives (e.g. benzylalcohol, phenol), and antioxidants. The preparation for injection is usually filled in appropriate ampoules.

The preparations obtained as described above are safe and low toxic, and can be administered to, for example, human and mammals (e.g. rats, mice, rabbits, sheep, pigs, cattle, cats, dogs, monkeys, etc.).

The dosage of the said compound or its salt differs depending on the target individual, target organ, symptoms, and administration method, etc. When it is administered orally, in general, for adults (60 kg body weight), about 0.1 - 100 mg per day, preferably about 1.0 - 50 mg per day, more preferably about 1.0 - 20 mg per day is administered.

When it is administered non-orally, the dosage per dosing differs depending on the target individual, target organ, symptoms, and administration method, etc. For example, in case of injection in general, for adults (60 kg body weight), it is desirable to intravenously inject about 0.01 - 30 mg per day, preferably about 0.1 - 20 mg per day, more preferably about 0.1 - 10 mg per day. Converting the dosage for 60 kg, the said compound or its salt can be administered to other animals.

[0014]

In this specification and drawings, the codes of bases and amino acids are according to IUPAC-IUB Commission on Biochemical Nomenclature or common codes in the art. The examples are shown below. For amino acids that may have the optical isomer, L form is presented unless it is specified.

[0015]

DNA : deoxyribonucleic acid

cDNA : complementary deoxyribonucleic acid

A : adenine

T : thymine

G : guanine

C : cytosine

[0016]

The SEQ ID NOs shown in the Sequence Listing of this Specification present the sequences below.

[SEQ ID NO: 1] Base sequence of cDNA containing human UCP-2 promoter region cloned in Example 1.

[SEQ ID NO: 2] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 3] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 4] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 5] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[0017]

[EXAMPLES]

The present invention is explained in detail below showing examples, but it is not intended to limit the cope of this invention to the description.

Escherichia coli transformant TOP10/pCR-ucp2p5'#1-10 obtained in the Example 1 described below was deposited with the Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human Technology (NIBH) as deposit number FERM BP-6587 on November 24, 1998 and with Institute for Fermentation, Osaka (IFO) as deposit number IFO 16219 on November 11, 1998.

The present invention is explained in detail below showing examples, but it is not intended to limit the cope of this invention to the description.

[0018]

Example 1 Cloning of human UCP-2 cDNA

Using 0.5 ng of human kidney cDNA (Clontech Laboratory, California, USA) as the template and the base sequence of base number 55 to 82: 5'-ATGGTTGGGTTCAAGGCCACAGATGTGCCC-3' of previously reported human UCP-2 cDNA [Gimeno, R. et al. (1997), Diabetes, Vol. 46, 900-906] and the base sequence complementary to base number 1300 to 1329: 5'-ATACAGGCCGATGCGGACAGAGGCAAAGCT-3' as oligonucleotide primers, human UCP-2 gene was amplified by PCR (after heating at 94°C for 5 min., a cycle consisting of heating at 94°C for 1 min, 55°C for 0.5 min and 72°C for 1.5 min was repeated 30 times, followed by heating at 72°C for 5 min), then inserted into pCR-blunt vector. Using this plasmid DNA carrying the insert as the template, oligonucleotide primers were prepared, and probes were prepared using PCR DIG probe synthesis kit (Boehringer-Mannheim Co.) following the attached instruction. Using the prepared probes, human

genomic DNA library (Clontech Laboratory, California, USA) in 3×10^6 phages was screened using nitrocellulose filters. Plaque hybridization was performed using DIG Easy hyb (Boehringer-Mannheim Co.), DIG Wash and Block Buffer Set (Boehringer-Mannheim Co.), and DIG nucleic acid detection kit (Boehringer-Mannheim Co.) following the attached instruction. As a result, eight positive clones were obtained from 3×10^6 phages. Of these clones, an inner primer of non-coding exon of previously reported human UCP-2 cDNA sequence [Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687] was synthesized (5'-CAAAGCTGCCAGTGGCTATCATGGCCCG-3'), and a clone containing the non-coding exon was detected by PCR using a primer containing EMBL3 sequence (5'-GACCGGTCGACCCAGATCTGGGTCGACCTG-3'), and a genomic clone containing the 5' upstream region of UCP-2 was obtained. From the genomic clone, 3.5 kbp fragment containing UCP-2 promoter region was prepared, and inserted into pCR-blunt vector (Invitrogen Co.), and transformant *E. coli* TOP/10 pCR-UCP2P5' #1-10 was prepared. After that, the restriction enzyme map was prepared and the base sequence was determined. The determined base sequence is shown in Figures 1 to 6. As shown in Figures 1 to 6, base number 2271 - 2326 and 3416 - 3505 were completely consistent with human UCP-2 cDNA (Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687). Furthermore, the terminal base sequences of the consistent regions were consistent with Shahnborn rule, which is the characteristic of intron-exon boundary base sequence, suggesting that the consistent base sequences are introns. A sequence likely to be CpG island (base number about 2070 - 2270), which is a characteristic of promoters without containing TATA-box sequence, was also confirmed upstream of the first exon. In the promoter sequence described above, PPRE (base number 284 - 296), which is the regulator sequence of promoters of fat cell-related genes, and three C/EBP binding sites (base number 1316 - 1320,

1364 - 1368, 1698 - 1692) were confirmed.

[0019]

Example 2 Examination of human UCP-2 gene promoter activity

To confirm the promoter activity of the cloned genomic DNA fragment, luciferase assay was performed. pGL3-Basic plasmid (Promega Co.) carrying firefly luciferase gene as the reporter gene was used for the vector. As the internal standard, pRL-SV40 plasmid (Promega Co.) expressing sea pansy luciferase under control of SV40 promoter was used.

EcoRI fragment (3.5 kb) was isolated from the genomic human UCP-2 DNA and blunted using Blunting High Kit (TOYOBO Co.), and then ligated to SmaI-digested pGL3-Basic plasmid DNA. Following the above procedure, human UCP-2 promoter/luciferase vector was constructed in which the base number 1 - 3505 shown in Figures 1 to 6 was inserted into pGL3-Basic vector. The constructed human UCP-2 promoter/luciferase vector was transiently transfected in HepG2, the human liver cancer cell line, in which constant expression of UCP-2 was confirmed by RT-PCR, and the activity was examined.

HepG2 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 60,000 cells/well, and cultured at 37°C in 5% CO_2 overnight. Using SuperFect Transfection Reagent (QIAGEN Co.), cells were transiently transfected with 1 μg of human UCP-2 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 μg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. Then, the cells were cultured at 37°C in 5% CO_2 for 24 hours, and the luciferase activity was detected using PicaGene Dual Sea Pansy (Nippon Gene Co.) according to the attached instruction. The measurement data were presented as relative activity to the internal standard value of pRL-SV40-derived sea pansy luciferase activity. The results are shown in Figure 7. The human UCP-2 promoter/luciferase

vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter. Therefore, the genomic DNA of human UCP-2 gene of this invention has a promoter activity reflecting the in vivo UCP-2 gene expression system.

[0025]

[Meritorious effects of the invention]

Since UCP-2 promoter of this invention contains the regulator sequence, it has higher activity reflecting the in vivo UCP-2 DNA expression system in human than the promoter lacking the regulator sequence. Therefore, the UCP-2 promoter of this invention can be used as a promoter inserted in vectors for treatment of human diseases and setting drug-screening systems under conditions closer to in vivo environment in human.

[0020]

[SEQUENCE LISTING]

[SEQ ID NO: 1]

Length: 3505

Type: DNA

Number of strands: double-stranded

Topology: linear

Sequence: cDNA

Sequence:

AACGGATCTG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT GCAGGCGTGA GCCACCTCAC 60

CTGGCTACAA GTTTTCAAAA TACATTTATC TAGTACCCAT ACATTCTCCA GTTTGTCCAC 120

AGGACATCTT ATGACTTGAG CAAGCTGCTA AAAATCCAAG GGTGCAGCGT TTGTATGTCT 180

ATAGGATTGC TCAGATCTGC CCCCACCCTG AAAGAATTTA AGAGAATTTC TTGAGGCCAG 240

GCACAGTGGC TCACACCTGT AATTCCAGTA CTGTGAGAGT CCGAGGTCAG AGGACTGCTT 300

GAGGCCAGGA GTTCAAGAGC AGCCTGGACA ACACAGGGAG ACCTGTCACT ACAAAGAATA 360

AATAAATTAG CCAGGCTTAG TGGCTCATCC CTGTGGTCCC AGCTACTAGG GAGGCAGAAG 420

TAGGACTGCT TGTCCCAGGA GGTCAAGACT GCAGTGAGCT GAGACCCAGC CACCTGCATT 480

CCAGCCTGGG CAACAAAAAG AGACCCTGTC TCAAAAAATA AGTTAAATAA ATAAATAATA 540

AAAATAGTTT AAACCCTAAA CACATCTTCT TTTTCAAAGA GGACTTCTTA AGGACTTCAT 600

GCTGCGTCCT GTTGATCTCC ACTTCCCTTT TTCAGCGTCC ACACTTTTAA CAGTCTCTTT 660

TGCCAAGGAT AATAAGTATA TAGTTTCTGG AATCCAGATT CTTCCCTGTT TGGACAGCCA 720

GGGGGACAAT TTTTGGTCTG CAGGCCTTTG CATCTGTTCT GCTGTTGCTC AGCAATCTCA 780

CAGCAAATTT GCCGAGCCTC TCCGGAATGC ACAGCCAGAC AGAGCTCAGC GCAAAAGCTA 840

GAGAACCTGG CGGAGGGAGA CTCACAGTGC CACAAAAAAA CTTTATCTTT TCTTTTTTTT 900

TTTCTTTTCT TTCTTTCTCT TTCTTTCTTG TCTTTCTGTC TTTCCTCTCT CTCTCTCTGT 960

CTTTCTTTCC TCTCTTTCTT TCTTTTTTCC TACATGGCAA GATCTCCTCA TGGCAGAAAT 1020

AATCTGCCTT GACTTCTGTT TCCACGCTGC TTCTGCCAGG ACCATGCGCT CGGCGTGTTT 1080

TTCTTTCCGC TATAATTATC CAGGCCCATC CCAGCTCTGG TCCCCTCAGC TGTTCCCTGG 1140

CAGTCCCTTC TGCTGGTGAA AACACATATG GCGCCGGCCT GACCAGGGTG TAAGTGTGTG 1200

AATATCAGGA AGATGACTGA ACGTCTTTGG GACTCCGTTT CCTCATTGTA AAATGGAGGT 1260

TAATACCAGC CTTCTTCTAC TCCCCAAACG CACGTGTTTG TCCCGGCCAG AGGGCCCAAT 1320

TGTTGGCTGT TCACGCATCA GTTACCCCCA CAGGACGGGT CAGCCAATTA AAGGCGAACC 1380

AGGCCCGGTC CATCTCCTGA CGCCTTTTCT CATCCCAGGG CTGGACAGGC AGCTGGCCTG 1440

GGCCCGGCTC TGCCTTGTCA CGTGCGGGGG CCGGCCCGTT TGCTTGTCTG TGTGTAGGAG 1500

CGTGAGGTCA CGCTGGGTGC TCCCGCCCCG CCGGGGCCTT TAGTGTCCCT GGTCCCTAAA 1560

CGCCAGGCCG CTCCACCGGG GGAGAAGGCG CGAACCCCAG CCGAGCCCAA CGGCTGTTGT 1620

CGGTTGCCGG GCCACCTGTT GCTGCAGTTC TGATTGGTTC CTTCCCCCGA CAACGCGGCG 1680

GCTGTAACCA ATCGACAGCG AGGCCGGTCG CGAGGCCCCA GTCCCGCCCT GCAGGAGCCA 1740

GCCGCGCGCT CGCTCGCAGG AGGGTGGGTA GTTTGCCCAG CGTAGGGGGG CTGGGCCCAT 1800

AAAAGAGGAA GTGCACTTAA GACACGGCCC CGCTGGACGC TTGTTAGAAA CCGTCCTGGC 1860

TGGGAAGGCA AGAGGTGTGT GACTGGACAA GACTTGTTTC TGGCGGTCAG TCTTGCCATC 1920

CTCACAGAGG TTGGCGGCCC GAGAGAGTGT GAGGCAGAGG CGGGGAGTGG CAAGGGAGTG 1980

ACCATCTCGG GGAACGAAGG AGTAAACGCG GTGATGGGAC GCACGGAAAC GGGAGTGGAG 2040

AAAGTCATGG AGAGAACCCT AGGCGGGGCG GTCCCCGCGG AAAGGCGGCT GCTCCAGGGT 2100

CTCCGCACCC AAGTAGGAGC TGGCAGGCCC GGCCCCGCCC CGCAGGCCCC ACCCCGGGCC 2160

CCGCCCCCGA GGCTTAAGCC GCGCCGCCGC CTGCGCGGAG CCCCACTGCG AAGCCCAGCT 2220

GCGCGCGCCT TGGGATTGAC TGTCCACGCT CGCCCGGCTC GTCCGACGCG CCCTCCGCCA 2280

GCCGACAGAC ACAGCCGCAC GCACTGCCGT GTTCTCCCTG CGGCTCGGTG AGCCTGGCCC 2340

CAGCCCTGCG CCCTTTGCGC CCCCCACGCT TGTTCTGCGT GCGCTGCCCG CTCTTCCATT 2400

TACCTTCTCT CCCACCCAAG TTTGTACTCT TTTCTTTCTC TCGGTTTTAT TTTTTGTTTT 2460

TGTTTGTTTG TTTGAGACAG GCTTTCGCTC TGTCTCCCAG GCTGGAGTGC AGTGGCGCGA 2520

TCTCGGCTCA CTGCAGCCTC CACCTCCCAG GTTCAAGCGA TCCGCCTGCC GAGTAGCTGG 2580

GATTACAGGC GCCCGCCACC ACGCCTGGCT AATTTTTGTG TTTTGTAGAG ATGGGGTTTC 2640

GCCATGTTGG CCAGGCTGGC CTCGAACTGC TCAGCTCAAG CAATCCGCCC GCCTCGGCCT 2700

CACAAAGTCC TAGAATTTTA GGCATGAGCC TCCGGGTCCG GCCTGTGCTA ATCCTTTCTG 2760

TCCTTGGTTC TTTATTTCCC TTCTCTCTTT TTCTTAGTCC CTTTTGTTCT TTCCCTCTCC 2820
CGTTCAGTTG GCTGTCGTTT GAGCCTCCAC CTTTTCACTC CCTCCTTTCC ACCACGATGC 2880
CGAGCCCTGC CTTGGATGGG GACCATCAGC GATGACCACA ATGACCTCTC CCTTACCAGG 2920
CAGCTCCAGG CAGTGTTCCT GCACCGCCTT TCCCAAGGCT TGGGGGCTTT TTCTAGTGGG 3000
CTTTGAGCTG CTCAATCTGG CCTCTGCAGG GCCGGCTCCC AGCCCTTCCA ACCTCCTCAC 3060
AGCCCGACCT GGGACCTAGC CAATTCCCGG AGAGTCTCTG TCCCATCGTG ACCCCCTCAC 3120
AACTCTCCCA CTCACCAAAG TCTGATGACT GTGCTAGGGG GTGCTTATAT AGAGTACTGA 3180
GTGTTACAAA AGCAGAAGTC TGGATGAGAA CCAATTTGTG ATATTAAGCA GGTGGGGTGG 3240
GGGTGGGGAG TGTACCTAGG TTCATTTTCC GCCCTGCTTT TCCCCTTTCC AGTGTGTGCA 3300
CTTAACCAGT CCCTGGGCCC TGTTCCCCAT CCCCCTCCAA GGCATGGATT GGGTGGGCTT 3360
GTGTGTCTTG GGGCAGGTGG CCCTTTCTAA ACTCTCTGCC TTTGCTCACC CACAGGACAC 3420
ATAGTATGAC CATTAGGTGT TTCGTCTCCC ACCCATTTTC TATGGAAAAC CAAGGGGATC 3480
GGGCCATGAT AGCCACTGGC AGCTT 3505

[0021]

[SEQ ID NO: 2]

Length: 30

Type: DNA

Number of strands: single-stranded

Topology: linear

Sequence: other nucleic acid, synthetic DNA

Sequence:

ATGGTTGGGT TCAAGGCCAC AGATGTGCCC 30

[0022]

[SEQ ID NO: 3]

Length: 30

Type: DNA

Number of strands: single-stranded

Topology: linear

Sequence: other nucleic acid, sybthetic DNA

Sequence:

ATACAGGCCG ATGCGGACAG AGGCAAAGCT 30

[0023]

[SEQUENCE LISTING]

[SEQ ID NO: 4]
Length: 28
Type: DNA
Number of strands: single-stranded
Topology: linear
Sequence: other nucleic acid, synthetic DNA
Sequence:
CAAAGCTGCC AGTGGCTATC ATGGCCCG 28

[0024]
[SEQUENCE LISTING]
[SEQ ID NO: 5]
Length: 30
Type: DNA
Number of strands: single-stranded
Topology: linear
Sequence: other nucleic acid, synthetic DNA
Sequence:
GACCGGTCGA CCCAGATCTG GGTCGACCTG 30

[0026]
[BRIEF DESCRIPTION OF THE DRAWINGS]

Figure 1 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued to Figure 2).

Figure 2 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 1 to Figure 2).

Figure 3 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 2 to Figure 4).

Figure 4 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 3 to Figure 5).

Figure 5 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1

(continued from Figure 4 to Figure 6).

Figure 6 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 5).

Figure 7 shows the luciferase activity measured in Example 2.

[Document] ABSTRACT

[Abstract]

[Problem to be solved]

This invention provides a DNA containing UCP-2 promoter region containing the regulator sequence.

[Means for solving problem]

This invention relates to a DNA containing uncoupling protein-2 (UCP-2) promoter region containing the regulator sequence, a transformant transformed with the said DNA, a method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of the said transformant, a method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of the said transformant, a kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of the said transformant, and pharmaceutical composition cntaining a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using the said screening method or the said screening kit.

[Meritorious effects]

Since UCP-2 promoter of this invention contains the regulator sequence, it has higher activity reflecting the in vivo UCP-2 expression system in human than the promoter lacking the regulator sequence. Therefore, the UCP-2 promoter of this invention can be used as a promoter inserted in vectors for treatment of human diseases and drug screening systems under conditions closer to in vivo environment in human.

[Document] Drawing

[Fig.1]

```
        10         20         30         40         50         60
AACGGATCTG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT GCAGGCGTGA GCCACCTCAC

        70         80         90        100        110        120
CTGGCTACAA GTTTTCAAAA TACATTTATC TAGTACCCAT ACATTCTCCA GTTTGTCCAC

       130        140        150        160        170        180
AGGACATCTT ATGACTTGAG CAAGCTGCTA AAAATCCAAG GGTGCAGCGT TTGTATGTCT

       190        200        210        220        230        240
ATAGGATTGC TCAGATCTGC CCCCACCCTG AAAGAATTTA AGAGAATTTC TTGAGGCCAG

       250        260        270        280        290        300
GCACAGTGGC TCACACCTGT AATTCCAGTA CTGTGAGAGT CCGAGGTCAG AGGACTGCTT
                                                  PPRE
       310        320        330        340        350        360
GAGGCCAGGA GTTCAAGAGC AGCCTGGACA ACACAGGGAG ACCTGTCACT ACAAAGAATA

       370        380        390        400        410        420
AATAAATTAG CCAGGCTTAG TGGCTCATCC CTGTGGTCCC AGCTACTAGG GAGGCAGAAG

       430        440        450        460        470        480
TAGGACTGCT TGTCCCAGGA GGTCAAGACT GCAGTGAGCT GAGACCCAGC CACCTGCATT
       490        500        510        520        530        540

CCAGCCTGGG CAACAAAAAG AGACCCTGTC TCAAAAAATA AGTTAAATAA ATAAATAATA
       550        560        570        580        590        600

AAAATAGTTT AAACCCTAAA CACATCTTCT TTTTCAAAGA GGACTTCTTA AGGACTTCAT

       610        620        630        640        650        660
GCTGCGTCCT GTTGATCTCC ACTTCCCTTT TTCAGCGTCC ACACTTTTAA CAGTCTCTTT
```

[Fig.2]

```
      670        680        690        700        710        720
TGCCAAGGAT AATAAGTATA TAGTTTCTGG AATCCAGATT CTTCCCTGTT TGGACAGCCA

      730        740        750        760        770        780
GGGGGACAAT TTTTGGTCTG CAGGCCTTTG CATCTGTTCT GCTGTTGCTC AGCAATCTCA
                                    GRE
      790        800        810        820        830        840
CAGCAAATTT GCCGAGCCTC TCCGGAATGC ACAGCCAGAC AGAGCTCAGC GCAAAAGCTA

      850        860        870        880        890        900
GAGAACCTGG CGGAGGGAGA CTCACAGTGC CACAAAAAAA CTTTATCTTT TCTTTTTTTT

      910        920        930        940        950        960
TTTCTTTTCT TTCTTTCTCT TTCTTTCTTG TCTTTCTGTC TTTCCTCTCT CTCTCTCTGT

      970        980        990       1000       1010       1020
CTTTCTTTCC TCTCTTTCTT TCTTTTTTCC TACATGGCAA GATCTCCTCA TGGCAGAAAT

     1030       1040       1050       1060       1070       1080
AATCTGCCTT GACTTCTGTT TCCACGCTGC TTCTGCCAGG ACCATGCGCT CGGCGTGTTT
  GRE
     1090       1100       1110       1120       1130       1140
TTCTTTCCGC TATAATTATC CAGGCCCATC CCAGCTCTGG TCCCCTCAGC TGTTCCCTGG

     1150       1160       1170       1180       1190       1200
CAGTCCCTTC TGCTGGTGAA AACACATATG GCGCCGGCCT GACCAGGGTG TAAGTGTGTG

     1210       1220       1230       1240       1250       1260
AATATCAGGA AGATGACTGA ACGTCTTTGG GACTCCGTTT CCTCATTGTA AAATGGAGGT
```

【Fig.3】

```
      1270       1280       1290       1300       1310       1320
TAATACCAGC CTTCTTCTAC TCCCCAAACG CACGTGTTTG TCCCGGCCAG AGGGCCCAAT
                                                          C/EBP

      1330       1340       1350       1360       1370       1380
TGTTGGCTGT TCACGCATCA GTTACCCCCA CAGGACGGGT CAGCCAATTA AAGGCGAACC
                                              C/EBP
      1390       1400       1410       1420       1430       1440
AGGCCCGGTC CATCTCCTGA CGCCTTTTCT CATCCCAGGG CTGGACAGGC AGCTGGCCTG
                                                    MyoD
      1450       1460       1470       1480       1490       1500
GGCCCGGCTC TGCCTTGTCA CGTGCGGGGG CCGGCCCGTT TGCTTGTCTG TGTGTAGGAG
           GRE
      1510       1520       1530       1540       1550       1560
CGTGAGGTCA CGCTGGGTGC TCCCGCCCCG CCGGGGCCTT TAGTGTCCCT GGTCCCTAAA

      1570       1580       1590       1600       1610       1620
CGCCAGGCCG CTCCACCGGG GGAGAAGGCG CGAACCCCAG CCGAGCCCAA CGGCTGTTGT

      1630       1640       1650       1660       1670       1680
CGGTTGCCGG GCCACCTGTT GCTGCAGTTC TGATTGGTTC CTTCCCCCGA CAACGCGGCG

      1690       1700       1710       1720       1730       1740
GCTGTAACCA ATCGACAGCG AGGCCGGTCG CGAGGCCCCA GTCCCGCCCT GCAGGAGCCA
         C/EBP
      1750       1760       1770       1780       1790       1800
GCCGCGCGCT CGCTCGCAGG AGGGTGGGTA GTTTGCCCAG CGTAGGGGGG CTGGGCCCAT

      1810       1820       1830       1840       1850       1860
AAAAGAGGAA GTGCACTTAA GACACGGCCC CGCTGGACGC TTGTTAGAAA CCGTCCTGGC

      1870       1880       1890       1900       1910       1920
TGGGAAGGCA AGAGGTGTGT GACTGGACAA GACTTGTTTC TGGCGGTCAG TCTTGCCATC
```

[Fig.4]

1930 1940 1950 1960 1970 1980
CTCACAGAGG TTGGCGGCCC GAGAGAGTGT GAGGCAGAGG CGGGGAGTGG CAAGGGAGTG

1990 2000 2010 2020 2030 2040
ACCATCTCGG GGAACGAAGG AGTAAACGCG GTGATGGGAC GCACGGAAAC GGGAGTGGAG

2050 2060 2070 2080 2090 2100
AAAGTCATGG AGAGAACCCT AGGCGGGGCG GTCCCCGCGG AAAGGCGGCT GCTCCAGGGT

2110 2120 2130 2140 2150 2160
CTCCGCACCC AAGTAGGAGC TGGCAGGCCC GGCCCCGCCC CGCAGGCCCC ACCCCGGGCC

2170 2180 2190 2200 2210 2220
CCGCCCCCGA GGCTTAAGCC GCGCCGCCGC CTGCGCGGAG CCCCACTGCG AAGCCCAGCT

2230 2240 2250 2260 2270 2280
GCGCGCGCCT TGGGATTGAC TGTCCACGCT CGCCCGGCTC GTCCGACGCG CCCTCCGCCA

2290 2300 2310 2320 2330 2340
GCCGACAGAC ACAGCCGCAC GCACTGCCGT GTTCTCCCTG CGGCTCGGTG AGCCTGGCCC

2350 2360 2370 2380 2390 2400
CAGCCCTGCG CCCTTTGCGC CCCCCACGCT TGTTCTGCGT GCGCTGCCCG CTCTTCCATT

2410 2420 2430 2440 2450 2460
TACCTTCTCT CCCACCCAAG TTTGTACTCT TTTCTTTCTC TCGGTTTTAT TTTTTGTTTT

2470 2480 2490 2500 2510 2520
TGTTTGTTTG TTTGAGACAG GCTTTCGCTC TGTCTCCCAG GCTGGAGTGC AGTGGCGCGA

2530 2540 2550 2560 2570 2580
TCTCGGCTCA CTGCAGCCTC CACCTCCCAG GTTCAAGCGA TCCGCCTGCC GAGTAGCTGG

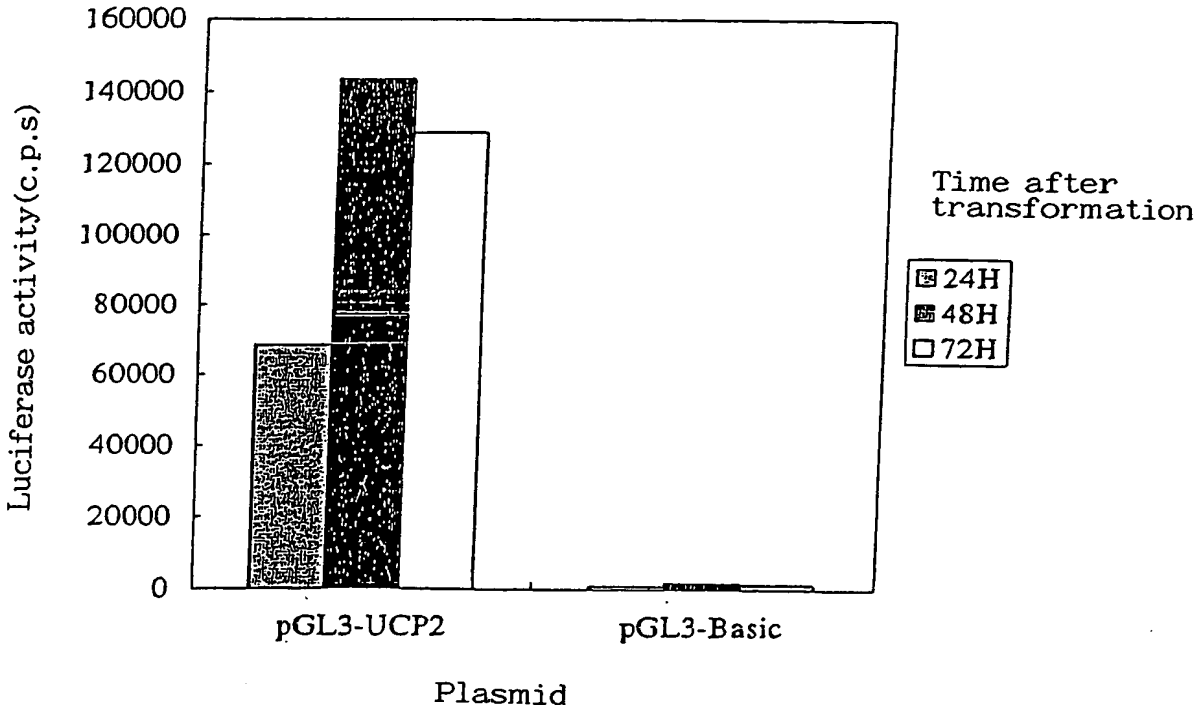
[Fig.5]

```
      2590       2600       2610       2620       2630       2640
GATTACAGGC GCCCGCCACC ACGCCTGGCT AATTTTTGTG TTTTGTAGAG ATGGGGTTTC

      2650       2660       2670       2680       2690       2700
GCCATGTTGG CCAGGCTGGC CTCGAACTGC TCAGCTCAAG CAATCCGCCC GCCTCGGCCT

      2710       2720       2730       2740       2750       2760
CACAAAGTCC TAGAATTTTA GGCATGAGCC TCCGGGTCCG GCCTGTGCTA ATCCTTTCTG

      2770       2780       2790       2800       2810       2820
TCCTTGGTTC TTTATTTCCC TTCTCTCTTT TTCTTAGTCC CTTTTGTTCT TTCCCTCTCC

      2830       2840       2850       2860       2870       2880
CGTTCAGTTG GCTGTCGTTT GAGCCTCCAC CTTTTCACTC CCTCCTTTCC ACCACGATGC

      2890       2900       2910       2920       2930       2940
CGAGCCCTGC CTTGGATGGG GACCATCAGC GATGACCACA ATGACCTCTC CCTTACCAGG

      2950       2960       2970       2980       2990       3000
CAGCTCCAGG CAGTGTTCCT GCACCGCCTT TCCCAAGGCT TGGGGGCTTT TTCTAGTGGG

      3010       3020       3030       3040       3050       3060
CTTTGAGCTG CTCAATCTGG CCTCTGCAGG GCCGGCTCCC AGCCCTTCCA ACCTCCTCAC

      3070       3080       3090       3100       3110       3120
AGCCCGACCT GGGACCTAGC CAATTCCCGG AGAGTCTCTG TCCCATCGTG ACCCCCTCAC

      3130       3140       3150       3160       3170       3180
AACTCTCCCA CTCACCAAAG TCTGATGACT GTGCTAGGGG GTGCTTATAT AGAGTACTGA

      3190       3200       3210       3220       3230       3240
GTGTTACAAA AGCAGAAGTC TGGATGAGAA CCAATTTGTG ATATTAAGCA GGTGGGGTGG
```

【Fig.6】

```
      3250       3260       3270       3280       3290       3300
GGGTGGGGAG TGTACCTAGG TTCATTTTCC GCCCTGCTTT TCCCCTTTCC AGTGTGTGCA

      3310       3320       3330       3340       3350       3360
CTTAACCAGT CCCTGGGCCC TGTTCCCCAT CCCCCTCCAA GGCATGGATT GGGTGGGCTT

      3370       3380       3390       3400       3410       3420
GTGTGTCTTG GGGCAGGTGG CCCTTTCTAA ACTCTCTGCC TTTGCTCACC CACAGGACAC

      3430       3440       3450       3460       3470       3480
ATAGTATGAC CATTAGGTGT TTCGTCTCCC ACCCATTTTC TATGGAAAAC CAAGGGGATC

      3490       3500       3510       3520       3530       3540
GGGCCATGAT AGCCACTGGC AGCTT..... .......... .......... ..........
```

【Fig.7】


160000
140000
120000
100000
80000
60000
40000
20000
0
Luciferase activity(c.p.s)
pGL3-UCP2
pGL3-Basic
Plasmid
Time after transformation
24H
48H
72H